PATENT COOPERATION TREATY

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To:

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PCT

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

(PCT Rule 71.1)

Date of mailing *(day/month/year)* 22.11.2005

Applicant's or agent's file reference
P34235A/CMU/MCM

IMPORTANT NOTIFICATION

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/GB2004/003391	05.08.2004	05.08.2003

Applicant
CSS-ALBACHEM LIMITED et al.

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary report on patentability and its annexes, if any, established on the international application.

2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.

3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.

4. **REMINDER**

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices) (Article 39(1)) (see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary report on patentability. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide.

The applicant's attention is drawn to Article 33(5), which provides that the criteria of novelty, inventive step and industrial applicability described in Article 33(2) to (4) merely serve the purposes of international preliminary examination and that "any Contracting State may apply additional or different criteria for the purposes of deciding whether, in that State, the claimed inventions is patentable or not" (see also Article 27(5)). Such additional criteria may relate, for example, to exemptions from patentability, requirements for enabling disclosure, clarity and support for the claims.

Name and mailing address of the international preliminary examining authority:

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PATENT COOPERATION TREATY

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INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

(Chapter II of the Patent Cooperation Treaty)

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Form PCT/IPEA/416
P34235A/CMU/MCM		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/GB2004/003391	05.08.2004	05.08.2003

International Patent Classification (IPC) or national classification and IPC

C07K1/107, C07K19/00

Applicant

CSS-ALBACHEM LIMITED et al.

1. This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of 5 sheets, including this cover sheet.
3. This report is also accompanied by ANNEXES, comprising:
   a. ☒ *sent to the applicant and to the International Bureau)* a total of 9 sheets, as follows:
      ☒ sheets of the description, claims and/or drawings which have been amended and are the basis of this report and/or sheets containing rectifications authorized by this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions).
      ☐ sheets which supersede earlier sheets, but which this Authority considers contain an amendment that goes beyond the disclosure in the international application as filed, as indicated in item 4 of Box No. I and the Supplemental Box.
   b. ☐ *(sent to the International Bureau only)* a total of (indicate type and number of electronic carrier(s)) , containing a sequence listing and/or tables related thereto, in computer readable form only, as indicated in the Supplemental Box Relating to Sequence Listing (see Section 802 of the Administrative Instructions).

4. This report contains indications relating to the following items:

☒	Box No. I	Basis of the opinion
☒	Box No. II	Priority
☐	Box No. III	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
☐	Box No. IV	Lack of unity of invention
☒	Box No. V	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
☐	Box No. VI	Certain documents cited
☐	Box No. VII	Certain defects in the international application
☐	Box No. VIII	Certain observations on the international application

Date of submission of the demand	Date of completion of this report
03.06.2005	22.11.2005

Name and mailing address of the International preliminary examining authority:

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INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/GB2004/003391

Box No. I Basis of the report

1. With regard to the **language**, this report is based on the international application in the language in which it was filed, unless otherwise indicated under this item.

   ☐ This report is based on translations from the original language into the following language , which is the language of a translation furnished for the purposes of:
   - ☐ international search (under Rules 12.3 and 23.1(b))
   - ☐ publication of the international application (under Rule 12.4)
   - ☐ international preliminary examination (under Rules 55.2 and/or 55.3)

2. With regard to the **elements*** of the international application, this report is based on *(replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report)*:

Description, Pages

1-57 as originally filed

Sequence listings part of the description, Pages

1-5 received on 24.11.2004 with letter of 23.11.2004

Claims, Numbers

1-27 received on 09.06.2005

Drawings, Sheets

1/15-15/15 as originally filed

☐ a sequence listing and/or any related table(s) - see Supplemental Box Relating to Sequence Listing

3. ☐ The amendments have resulted in the cancellation of:
   - ☐ the description, pages
   - ☐ the claims, Nos.
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

4. ☐ This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).
   - ☐ the description, pages
   - ☐ the claims, Nos.
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

* *If item 4 applies, some or all of these sheets may be marked "superseded."*

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/GB2004/003391

Box No. II Priority

1. ☒ This report has been established as if no priority had been claimed due to the failure to furnish within the prescribed time limit the requested:
   - ☒ copy of the earlier application whose priority has been claimed (Rule 66.7(a)).
   - ☐ translation of the earlier application whose priority has been claimed (Rule 66.7(b)).
2. ☐ This report has been established as if no priority had been claimed due to the fact that the priority claim has been found invalid (Rule 64.1). Thus for the purposes of this report, the international filing date indicated above is considered to be the relevant date.
3. Additional observations, if necessary:

Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-27
	No:	Claims	
Inventive step (IS)	Yes:	Claims	1-27
	No:	Claims	
Industrial applicability (IA)	Yes:	Claims	1-27
	No:	Claims	

2. Citations and explanations (Rule 70.7):

see separate sheet

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY (SEPARATE SHEET)

International application No.

PCT/GB2004/003391

Re Item V

Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. The present application refers to methods for producing oligopeptide products wherein a first oligopeptide product and a second oligopeptide / label molecule are linked via a linking moiety having formula I, formula II or formula III. The application also refers to labelled oligopeptides produced by such methods.

2. Reference is made to the following documents :

   D1: PERLER F.B. ET AL.: "The mechanism of protein splicing: variations on a theme" PEPTIDES 2002, 2002, pages 254-255, NAPOLI, ITALY

   D2: CHONG S ET AL: "Single-column purification of free recombinant proteins using a self-cleavable affinity tag derived from a protein splicing element" GENE: AN INTERNATIONAL JOURNAL ON GENES AND GENOMES, ELSEVIER SCIENCE PUBLISHERS, BARKING, GB, vol. 192, no. 2, 1997, pages 271-281.

   D3: COTTON GRAHAM J ET AL: "Peptide ligation and its application to protein engineering" CHEMISTRY AND BIOLOGY (LONDON), vol. 6, no. 9, September 1999 (1999-09), pages R247-R256.

   D4: WO 00/18881 A (XU MING QUN ; NEW ENGLAND BIOLABS INC (US); EVANS THOMAS C (US)) 6 April 2000 (2000-04-06)

   D5: GEOGHEGAN K F: "Site-directed conjugation of nonpeptide groups to peptides and proteins via periodate oxidation of a 2-amino alcohol. Application to modification at N-terminal serine" BIOCONJUGATE CHEMISTRY, AMERICAN CHEMICAL SOCIETY, WASHINGTON, US, vol. 3, no. 2, 1992, pages 138-146.

3. **Novelty; article 33(2) PCT.**

   The subject-matter of claims 1-27 has never been disclosed in the documents cited in the International Search Report (ISR). Therefore, claims 1-27 have to be considered as novel in the sense of Article 33(2) PCT.

4. Inventive step; article 33(3) PCT.

The documents D1 to D4 disclose the use of peptides linked to a modified intein for the generation of peptides having an activated C-terminal α thioester. This technique has been used for Expressed Protein Ligation or Intein-mediated Protein Ligation where the second peptide possesses a N-terminal cysteine residue which reacts with the thioester to form a peptide bond.

Even of the documents D1 and D4 refer to a general nucleophilic attack, all the examples disclosed in said documents involve the attack of the C-terminal thioester of a recombinant peptide by a peptide having a N-terminal cysteine.

None of the documents cited in the International Search Report suggest the methods and products of the present application

Therefore, the subject-matter of claims 1-27 has to be considered as inventive in the sense of article 33(3) PCT.

Claims

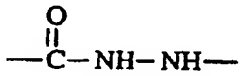
1. A method of producing an oligopeptide product, the method comprising the steps:

a) providing a first oligopeptide, the first oligopeptide having a reactive moiety,

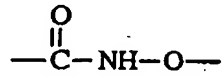
b) providing a second oligopeptide, the second oligopeptide having a activated ester moiety

c) allowing the reactive moiety of the first oligopeptide to react with the activated ester moiety of the second oligopeptide to form an oligopeptide product, in which the first and second oligopeptides are linked via a linking moiety having Formula I, Formula II or Formula III.

Formula I



Formula II



Formula III

2. The method according to claim 1 wherein the terminal activated ester moiety is a thioester wherein the peptide is the acyl substituent of

the thioester.

3. The method according to claim 2, wherein said second polypeptide is generated by thiol reagent dependent cleavage of a precursor molecule, said precursor molecule comprising a second oligopeptide fused N-terminally to an intein domain.

4. A method of producing an oligopeptide product, the method comprising the steps:
a) providing a first oligopeptide, the first oligopeptide having a reactive moiety,
b)i) providing a precursor oligopeptide molecule, the precursor oligopeptide molecule comprising a second oligopeptide fused N-terminally to an intein domain
ii) allowing thiol reagent dependent cleavage of the precursor molecule to generate a second oligopeptide molecule, said second oligopeptide molecule having a thioester moiety at its C-terminus,
c) allowing the reactive moiety of the first oligopeptide to react with the second oligopeptide molecule to form an oligopeptide product, in which the first and second oligopeptides are linked via a linking moiety having Formula I, II or III.

5. The method according to any one of the preceding claims wherein the reactive moiety is a hydrazine moiety, a hydrazide moiety or an aminooxy moiety.

6. The method according to claim 5, wherein the reactive moiety is an aminooxy moiety and the

activated ester moiety is a thioester.

7. The method according to claim 5, wherein said first oligopeptide is produced by reaction of hydrazine with a precursor molecule, said precursor molecule comprising a precursor oligopeptide fused N-terminally to an intein domain via a thioester moiety.

8. A method of producing an oligopeptide product, said method comprising the steps:
a) providing a first oligopeptide, the first oligopeptide having a reactive moiety, wherein the reactive moiety is a hydrazine moiety, a hydrazide moiety or an amino-oxy moiety;
b) providing a precursor oligopeptide molecule, the precursor oligopeptide molecule comprising a second oligopeptide fused N-terminally to an intein domain;
c) allowing the reactive moiety of the first oligopeptide to react with the precursor oligopeptide molecule to form an oligopeptide product, in which the first and second oligopeptides are linked via a linking moiety having Formula I, Formula II or Formula III.

9. The method according to any one of the preceding claims, wherein the first oligopeptide or the second oligopeptide is a recombinant oligopeptide and the other of the the first oligopeptide and the second oligopeptide is a synthetic polypeptide.

10. The method according to any one of claims 1 to 8, wherein the first oligopeptide and the second oligopeptide are recombinant oligopeptides.

11. The method according to any one of claims 1 to 8, wherein the first oligopeptide and the second oligopeptide are synthetic oligopeptides.

12. A method of generating a protein hydrazide, said method comprising the steps:
(a) providing a protein molecule comprising an oligopeptide fused N-terminal to an intein domain,
(b) reacting said protein molecule with hydrazine, such that the intein domain is cleaved from the oligopeptide to generate a protein hydrazide.

13. The method according to any one of the claims 1 to 11 wherein step (c) of the method is performed at a pH in the range pH 6.5 to 7.5.

14. A method of producing an oligopeptide product, the method comprising the steps:
a) providing a first oligopeptide, the first oligopeptide having an aldehyde or ketone moiety,
b) providing a precursor oligopeptide molecule, the precursor oligopeptide molecule comprising a second oligopeptide fused N-terminally to an intein domain,
c) reacting said precursor oligopeptide molecule

with hydrazine to generate an oligopeptide molecule comprising an intermediate oligopeptide, said intermediate oligopeptide having a terminal hydrazide moiety,

d) allowing the aldehyde or ketone moiety of the first oligopeptide to react with the hydrazide moiety of the intermediate oligopeptide molecule to form an oligopeptide product, in which first oligopeptide and the second oligopeptide are linked via a hydrazone linking moiety.

15. An oligopeptide product produced by the method of any one of the preceding claims, in which the first and second oligopeptides are linked via a linking moiety having Formula II or Formula III.

16. A method of labelling an oligopeptide, the method comprising the steps:

a) providing a label molecule, the label molecule having a reactive moiety,

b) providing the oligopeptide, the oligopeptide having a activated ester moiety

c) allowing the reactive moiety of the label molecule to react with the activated ester moiety of the oligopeptide to form the labelled oligopeptide, in which the label molecule and the oligopeptide are linked via a linking moiety having Formula I, Formula II or Formula III.

17. The method according to claim 16, wherein in step (c), where said label molecule and the oligopeptide are linked via a linking moiety

having Formula II and where said activated ester moiety of step (b) is not a thioester, said activated ester is a terminal activated ester moiety.

18. A method of labelling an oligopeptide, the method comprising the steps:
a) providing a label molecule, the label molecule having an activated ester moiety of which the label is the acyl substituent,
b) providing the oligopeptide, the oligopeptide having a reactive moiety
c) allowing the activated ester moiety of the label molecule to react with the reactive moiety of the oligopeptide to form the labelled oligopeptide, in which the label molecule and the oligopeptide are linked via a linking moiety having Formula I, Formula II or Formula III, wherein, in step (c), where said label molecule and the oligopeptide are linked via a linking moiety having Formula II and where said activated ester moiety of step (b) is not a thioester, said activated ester is a terminal activated ester moiety.

19. The method according to claim 18 wherein said oligopeptide is produced by reaction of hydrazine with a precursor molecule, said precursor molecule comprising a precursor oligopeptide fused N-terminally to an intein domain via a thioester moiety.

20. A method of labelling an oligopeptide, the method comprising the steps:
a) providing a label, the label having a reactive moiety,
b) (i) providing a precursor oligopeptide molecule, the precursor oligopeptide molecule comprising an oligopeptide fused N-terminally to an intein domain
(ii) allowing thiol reagent dependent cleavage of the precursor molecule to generate the oligopeptide molecule, said oligopeptide molecule having a thioester moiety at its C-terminus,
c) allowing the reactive moiety of the label to react with the oligopeptide molecule to form a labelled oligopeptide, in which the label and oligopeptide are linked via a linking moiety having Formula I, II or III.

21. The method according to any one of claims 16 to 18, wherein the reactive moiety is an aminooxy moiety and the activated ester moiety is a thioester.

22. The method according to claim 20, wherein the reactive moiety is an aminooxy moiety.

23. A method of labelling an oligopeptide, the method comprising the steps:
a) providing a label molecule, the label molecule having a reactive moiety,
b) providing a precursor oligopeptide molecule, the precursor oligopeptide molecule comprising an

oligopeptide fused N-terminally to an intein domain,

c) allowing the reactive moiety of the label molecule to react with the precursor oligopeptide molecule to form a labelled oligopeptide product, in which the label molecule and the oligopeptide are linked via a linking moiety having Formula I, Formula II or Formula III as defined above.

24. The method according to any one of claims 16 to 23 wherein step (c) of the method is performed at a pH in the range pH 6.5 to pH 7.5.

25. A method of labelling an oligopeptide, the method comprising the steps:

a) providing a label molecule, the label molecule having a aldehyde or ketone moiety,

b) providing a precursor oligopeptide molecule, the precursor oligopeptide molecule comprising a first oligopeptide fused N-terminally to an intein domain,

c) reacting said precursor oligopeptide molecule with hydrazine to generate an oligopeptide molecule comprising an intermediate oligopeptide, said intermediate oligopeptide having a terminal hydrazide moiety,

d) allowing the aldehyde or ketone moiety of the label molecule to react with the hydrazide moiety of the intermediate oligopeptide molecule to form a labelled oligopeptide product, in which the label molecule and oligopeptide are linked via a

hydrazone linking moiety.

26. The method according to claim 14 or claim 25, wherein the aldehyde or ketone moiety is an α-diketone or an α-keto-aldehyde group.

27. A labelled oligopeptide produced by the method of any one of claims 16 to 26, in which the first and second oligopeptides are linked via a linking moiety having Formula II or Formula III.